Reduced Production, Absorption, and Elimination of Erythropoietin in Uremia Compared With Healthy Volunteers

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(1) The purpose of this study was to investigate the metabolism of erythropoietin (EPO) in uremia compared with healthy subjects. Twenty-one patients (nine men and 12 women) with end-stage renal failure and anemia and 12 healthy volunteers (3 women and nine men) were studied. The pharmacokinetic parameters were calculated after an iv and a femoral sc injection of 100 U/kg of recombinant human EPO. The serum EPO (s-EPO) was measured by radioimmunoassay at regular intervals until 48 h (iv) and 120 h (sc). In uremia, the median terminal elimination half-life was significantly longer (8.31 versus 4.92 h; \( P < 0.001 \)) and the clearance was reduced (5.00 versus 7.88 mL/min per 1.73 m\(^2\); \( P < 0.01 \)). The volume of distribution was (3.70 versus 3.31 L/1.73 m\(^2\)) not significant. The estimated endogenous EPO production was significantly lower in uremia (146 versus 290 U/day per 1.73 m\(^2\); \( P < 0.001 \)). After sc administration, the bioavailability was significantly lower in the patients (23.7 versus 38.5%; \( P < 0.01 \)), and the maximal s-EPO was lower (113 versus 153 U/L; \( P < 0.05 \)) and delayed (15.4 versus 11.0 h; \( P < 0.02 \)), but the mean input time (sc) was not significantly different (23.3 versus 27.8 h). The basal s-EPO was lower in the uremic patients (20.0 versus 26.3 U/L; \( P < 0.05 \)). There was no difference between patients treated with hemodialysis and peritoneal dialysis or between uremic men and women. There was no correlation between the pharmacokinetic parameters and age. It was concluded that uremia was associated with a reduced production rate of EPO, a reduced sc bioavailability, and a prolonged elimination of exogenous EPO compared with healthy volunteers. The low clearance of EPO in uremia may help to preserve a normal basal level of s-EPO, even in the presence of a reduced production of EPO.

Key Words: Dialysis, recombinant human erythropoietin, erythropoietin administration, intravenous, subcutaneous, pharmacokinetics, erythropoietin metabolism

Erythropoietin (EPO) is produced mainly in the adult kidney and to some extent extrarenally (1,2), in the liver (3). Animal experiments suggest that the liver (4), the hemopoietic cells (5,6), and the kidney (7,8) itself may take part in the elimination. The only human study comparing the influence of renal insufficiency on the metabolism of EPO revealed no significant differences, but it included only two healthy control subjects (9). The EPO production rate was normal in uremic patients, as measured by means of constant infusion of \(^{125}\)I-EPO tracer, but the clearance was not reported (10). Animal experiments have shown that renal failure can be accompanied by a reduction of the elimination of EPO (11,12).

We compared the pharmacokinetics of iv and sc administered EPO in uremic patients and healthy volunteers. The aim was to clarify the influence of renal failure on the production rate, absorption rate, bioavailability, elimination rate, and distribution volume of EPO.

MATERIALS AND METHODS

Study Subjects

The main clinical and laboratory data of the patients and the healthy volunteers are listed in Table 1. The duration of dialysis before the study was (median) 4.2 (range, 0.4 to 18) yr. Thirteen patients were treated with hemodialysis (HD), seven were treated with continuous ambulatory peritoneal dialysis (CAPD), and 1 was resuming CAPD at the time of entrance in the study because of chronic renal graft failure. The original renal disease was chronic glomerulonephritis in 12, chronic interstitial nephritis in 2, diabetic nephropathy in 3, congenital hypoplasia in 1, malignant nephrosclerosis in 1, and...
unknown ESRD in 2 patients. Three patients were anephric; three had a nonfunctioning renal allograft in situ but no native kidneys. Fifteen patients had their native kidneys in situ and no renal allografts. Most of the patients had received multiple blood transfusions. The transferrin saturation was 37% (range, 8 to 10%), and the serum ferritin was 516 ng/L (range, 8 to 9,550 ng/L). In the uremic patients, the serum albumin was 39 g/L (27 to 44). and the serum alkaline phosphatase was 1.8 U/L (95 to 359), the serum bilirubin was 4 µmol/L (2 to 7), and the serum aspartate aminotransferase was 20 U/L (7 to 57), the serum hematocrit was 42 (35–47), and the serum creatinine was 93 (58–107) µmol/L.

### Methods

s-EPO was determined by a radioimmunoassay based entirely on recombinant EPO. The EPO antiserum was obtained from rabbits immunized with human recombinant EPO (epoetin beta; Boehringer Mannheim). The standard was made from the same recombinant EPO and calibrated with the second international reference preparation (13). One hundred microliters of unknown sample or standard (0 to 1,000 U/mL) and 100 µL of antiserum (dilution of 1:15,000) were added to 200 µL of assay buffer (phosphate-buffered saline, pH 7.4, with 0.2% human serum albumin) in duplicate and incubated for 24 h at 4°C. Then, 100 µL of 125I-labeled recombinant EPO tracer (Amersham Int., United Kingdom) was added (approximately 4,000 cpm/tube) and incubated for another 48 h at 4°C. Three hundred microliters of solution with a second antibody coupled with iron activity. The dose of EPO was 100 U/kg at both occasions. At sc injection, the dose was dissolved in 1 mL of water, and a 1-mL insulin syringe with a 0.4 × 12 mm needle (Myojector®; Terumo, Belgium) was used. At both examinations, each individual received EPO from the same batch. After iv injection, serum EPO (s-EPO) was measured at regular intervals at 0, 2, 5, 10, 15, and 30 min and after 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, and 48 h. After sc injection, the s-EPO was measured at 0, 15, and 30 min and after 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, 48, 72, 96, and 120 h. All of the investigations were initiated at 9:00 a.m. The patients treated with intermittent HD were investigated the day before dialysis. The patients treated with CAPD received their EPO injection just after the dialysis bag was changed. Subsequently, four shifts of dialysis bags (2 L) were performed during the next 24 h. The iv pharmacokinetic procedure was conducted in all of the subjects. The sc procedure was performed in 20 of 21 of the uremic patients and in 8 of 12 of the healthy volunteers (male/female, 5/3; age, 27 (24 to 37) yr).

### Procedure

The study was performed consecutively before the uremic patients entered regular treatment with EPO. Each pharmacokinetic study was performed over two visits. At the first visit, each patient received an iv injection of EPO (Recormon®; Boehringer Mannheim, Germany) in a brachial vein. At the second visit, 1 to 2 wk later, the same person received an sc injection of EPO in the anterior femoral skin. The study subjects were kept at rest for the first hour after the injection and were treated as inpatients for the first 24 h. After that time, they were doing their everyday activities.

### TABLE 1. Number, age, body weight and height, and blood pressure of the study subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Volunteers</th>
<th>Uremia Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N, Sex (Male/Female)</strong></td>
<td>9/3</td>
<td>9/12</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>30 (24–56)</td>
<td>42 (22–66)</td>
</tr>
<tr>
<td><strong>Body Wt (kg)</strong></td>
<td>72.2 (53.9–100.0)</td>
<td>62.0 (43.9–96.5)</td>
</tr>
<tr>
<td><strong>Body Ht (cm)</strong></td>
<td>179 (159–186)</td>
<td>171 (150–190)</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (mm Hg)</strong></td>
<td>120 (107–140)</td>
<td>135 (78–180)</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mm Hg)</strong></td>
<td>79 (50–95)</td>
<td>80 (48–105)</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td>42 (35–47)</td>
<td>25 (19–38)</td>
</tr>
<tr>
<td><strong>Serum Creatinine (µmol/L)</strong></td>
<td>93 (68–107)</td>
<td>955 (487–1,460)</td>
</tr>
</tbody>
</table>

* Values are median (range).
(donkey anti-rabbit antiserum; Amerlex-M; Amer sham Int.) was added, and the bound fraction of radioactivity was precipitated on a magnetic plate, washed with assay buffer, and counted. Nonspecific binding was 1% (mean) of total counts. Precipitated activity in the zero standard was 30%. The detection limit was 0.4 mU/tube. The coefficients of variation were 7.7% (interassay) and 4.2% (intra-assay). The assay did not discriminate between endogenous EPO and recombinant EPO.

**Gel Chromatography**

Gel chromatography was performed on Sephadex G 150 (Pharmacia, Sweden) columns, 56 cm in height and 1 cm in diameter. The sample volume was 2.5 mL. The flow rate was 6 mL/h. Fractions were collected in volumes of 0.6 mL and were assayed in the radioimmunoassay. The presence of circulating immunoreactive degradation fragments of EPO was investigated in samples from normal and uremic subjects 16 h after iv EPO.

**Pharmacokinetic Calculations**

The basal endogenous s-EPO level was considered to be constant during each pharmacokinetic investigation period. The area under the curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoid method. Mean residence time (MRT) was calculated as AUMC/AUC. The mean input time (MIT) was calculated as MIT = MRTrev - MRTiv. Clearance = Dose/AUCrev where Dose is the injected dose and AUCrev is the area under the curve after iv injection. The bioavailability or absorption fraction was calculated as f = AUCrev/AUCiv. The time (t_m) of maximal s-EPO (C_m) after sc injection was determined. The s-EPO concentration versus time curve was subjected to nonlinear regression analysis according to first-order absorption C = [(f × a × Dose/V) × (e^{-axt} - e^{-ax0})]. In each patient, β was calculated from the iv concentration curve and was assumed to be constant for the estimation of the absorption rate a and the t_m = ln 2/a from the sc curve.

**Statistics**

Nonparametric tests were used for statistical analysis. Mann-Whitney’s rank sum test was used for the comparison of two groups. Wilcoxon’s test was used for paired analysis. Spearman’s test was used for the calculation of correlations. P = 0.05 was considered as the limit of significance. The results are given as the median (range) unless otherwise stated.

**RESULTS**

The concentration versus time curves for iv-injected EPO is presented in Figure 1. After 6 h, the s-EPO level was highest in the uremic group. The principal pharmacokinetic results derived from the iv data are listed in Table 2. According to the criteria,

![Figure 1](https://example.com/figure1.png)

**Figure 1.** s-EPO versus time curve for uremic patients (open circles) and healthy volunteers (closed circles) after iv injection of recombinant human EPO (100 U/kg). Mean ± SD.
TABLE 2. Principal pharmacokinetic parameters from iv administration of EPO

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Volunteers</th>
<th>Uremia Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (U/h L)</td>
<td>13,532 (9,412-22,686)</td>
<td>20,639 (8,632-29,832)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$t_{1/2a}$ (h)</td>
<td>2.67 (0.17-3.48)</td>
<td>1.36 (0.18-4.50)</td>
<td>N=4/10</td>
</tr>
<tr>
<td>$t_{1/2b}$ (h)</td>
<td>4.92 (3.58-9.20)</td>
<td>8.31 (4.38-13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clearance (ml/min per 1.73 m$^2$)</td>
<td>7.93 (5.45-11.04)</td>
<td>5.00 (3.65-13.26)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Volume of Distribution (V) (L/1.73 m$^3$)</td>
<td>3.14 (2.60-6.00)</td>
<td>3.82 (2.15-7.76)</td>
<td>NS</td>
</tr>
<tr>
<td>Volume of Distribution (Vs) (L/1.73 m$^3$)</td>
<td>3.31 (2.65-7.09)</td>
<td>3.70 (2.24-7.87)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Residence Time (h)</td>
<td>7.8 (6.0-10.7)</td>
<td>11.6 (6.4-16.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Basal s-EPO (U/L)</td>
<td>26.3 (15.7-38.9)</td>
<td>20.0 (6.8-47.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Native EPO Production (U/day per 1.73 m$^2$)</td>
<td>290 (175-427)</td>
<td>146 (64-647)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* $t_{1/2a}$, half-life of distribution in to-compartment model (healthy volunteers, N = 3: uremic patients, N = 10. $t_{1/2b}$ terminal half-life of elimination. Volume of distribution, V in β phase and = Vs at steady state. Values are median (range). Mann-Whitney’s test. NS, not significant.

10 of 21 uremic patients and 4 of 10 healthy subjects were best fitted to a two-compartment model. The $t_{1/2a}$ was significantly longer in the uremic patients than in the healthy subjects, corresponding to the significantly reduced clearance in the uremic group. The V and Vs were not significantly different. The calculated native EPO production in chronic renal failure was approximately half the level found in the healthy persons. The excretion in the urine and the peritoneal dialysis fluid was negligible, accounting for 1% or less of the iv administered dose during the first 24 h.

Figure 2 shows the sc concentration versus time curves. The s-EPO level was higher in healthy individuals in the interval between 2 and 24 h and lower after 48 h. The principal pharmacokinetic results or sc data are listed in Table 3. The bioavailability after sc injection was considerably lower in the renal failure group compared with the healthy group. The $t_{1/2a}$ estimated by regression analysis was not different in the two groups. This is in agreement with the calculated MIT. The $C_m$ was lower and delayed in the uremic patients. Some of the patients had a dual peak pattern at 12 h (9:00 p.m.) and 24 h (9:00 a.m.), with a local minimum at 18 h (3:00 a.m.).

The reduced number of healthy subjects that completed the sc part of the study were younger than the uremic patients. Therefore, the uremic patients were divided by age in two groups of 10 patients each; one group was aged 22 to 42 yr, and another group was aged 45 to 66 yr. The male/female ratio was 4/6 in both groups. The sc data from the group of young uremic patients were compared with data from the healthy subjects (medians): AUC = 4,627 versus 5,210 U x h/L (not significant) bioavailability = 20.8 versus 38.5% (P < 0.05), MIT = 41.3 versus 30.2 h (P < 0.05), $t_{1/2a}$ = 19.8 versus 11.0 (P < 0.06); $C_m$ = 109 versus 153 U/L (P < 0.05). The pharmacokinetic parameters (iv and sc) in the groups of younger and older patients were not significantly different.

There was no significant difference in any of the parameters between HD- and CAPD-treated patients (median $t_{1/2a}$ 7.9 versus 8.3 h; clearance, 6.1 versus 4.5 mL/min per 1.73 m$^2$; production rate, 158 versus 120 U/day per 1.73 m$^2$; and bioavailability, 25.1 versus 23.8%) or between male and female patients (median $t_{1/2a}$ 7.7 versus 9.0 h; clearance, 5.8 versus 6.0 mL/min per 1.73 m$^2$; and bioavailability, 25.1 versus 23.8%).
TABLE 3. Principal pharmacokinetic parameters from sc administration of EPO

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Volunteers</th>
<th>Uremia Patients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC U/h-L</td>
<td>5,210 (1,814–8,103)</td>
<td>4,560 (2,090–11,529)</td>
<td>NS</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>38.5 (9.0–60.3)</td>
<td>23.7 (11.3–48.7)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>22.58 (10.3–26.76)</td>
<td>21.7 (10.5–40.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Residence Time (h)</td>
<td>30.2 (17.5–44.5)</td>
<td>38.8 (24.8–67.6)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Mean Input Time (h)</td>
<td>23.3 (10.3–36.5)</td>
<td>27.8 (10.3–52.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal s-EPO (U/L)</td>
<td>153 (106–358)</td>
<td>143 (81–387)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Time at Maximal s-EPO (h)</td>
<td>11.0 (8.0–24.0)</td>
<td>15.4 (8.0–24.0)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

* t<sub>1/2</sub>, estimated half-life of absorption. Values are median (range). Mann-Whitney’s test. NS, not significant.

4.8 mL/min per 1.73 m²; production rate, 148 versus 140 U/day per 1.73 m²; and, bioavailability, 23.5 versus 24.3%). Likewise, the results from the male healthy volunteers (median t<sub>1/2</sub>, 5.0 h; clearance, 8.0 mL/min per 1.73 m²; production rate, 281 U/day per 1.73 m²; and bioavailability, 39.1%) were significantly different from those of the male uremia patients. The group without native kidneys with or without a nonfunctioning renal graft was not significantly different in any of the pharmacokinetic parameters (median t<sub>1/2</sub>, 8.5 h; clearance, 6.4 mL/min per 1.73 m²; and production rate, 187 U/day per 1.73 m²). The three totally anephric patients had a t<sub>1/2</sub> of 6.1 to 9.8 h, a clearance of 4.9 to 13.3 mL/min per 1.73 m², and a production rate of 146 to 647 U/day per 1.73 m². There was no correlation between age and the pharmacokinetic parameters in the patients or the healthy subjects. A moderate but significant positive correlation was shown between hematocrit and basal s-EPO level in the uremic patients (r = 0.53; P < 0.02), but not in the healthy individuals (r = –0.47; P < 0.12).

Figure 3 shows the gel chromatography of the pure recombinant EPO compared with immunoreactive EPO in serum samples taken 16 h after iv-administered EPO from healthy and uremic subjects. No immunoreactive split fragments were identified.

**DISCUSSION**

This study showed reduced bioavailability, elimination rate, clearance, and native EPO production in chronic renal failure. The estimated sc absorption rate and volume of distribution were comparable to those of healthy individuals.

A number of pharmacokinetic studies of EPO in humans have been published. The range of the t<sub>1/2</sub> of elimination in these studies was 4.9 to 11.2 h (9,15–25), and the range of clearance was 2.8 to 10.1 mL/kg per hour (9,20,23,25), 5.7 to 11.7 mL/min (15,17,18,21,24), and 6.0 to 7.8 mL/min per 1.73 m² (22) in uremic patients. Nearly the same level has been reported in healthy men: t<sub>1/2</sub> of elimination = 4.4 to 11.0 h and clearance = 4.1 to 14.7 mL/kg per hour (26–29). In humans, only one comparative study has been performed to investigate the effect of renal insufficiency on the pharmacokinetics of EPO. In that study, two healthy individuals were compared with eight patients with different degrees of renal insufficiency. No differences appeared after an iv bolus injection of EPO (9).

Direct comparison between these investigations is difficult. There may be differences in assays for EPO measurements, timing of blood samples, model-dependent or -independent calculations, dose of EPO, whether or not the results are standardized to body size, and variations between different batches of EPO from the manufacturers. Differences between epitope α and β have been reported in healthy men (29) but not in dialysis patients (19). In this investigation, all of the procedures were the same in the two groups. The choice of model was determined by predefined criteria, and the pharmacokinetic results were confirmed by both model-dependent and -independent parameters. The same dose and injection site were used in all of the study subjects, and the same batch of EPO was used for iv and sc investigations.

**In vitro** experiments have shown that the renal parenchyma from rats was able to degrade EPO (30). Total nephrectomy resulted in a reduced t<sub>1/2</sub> of elimination in rats (11,31) and in dogs (12). Other investigators did not find this effect in rats (32) or sheep (33). Subtotal (%) nephrectomy delayed the elimination of EPO in rabbits (34), as did the ligation of the renal pedicles in rats (30) but not the ligation of the ureters (11). Some of these studies seem to indicate that surgical reduction or exclusion from the circulation of the renal parenchyma rather than the uremia itself may be the cause of the delayed elimination of EPO. The sources of EPO were from various species and of variable degrees of purity. Besides, the results may not be directly transferable to humans. In our study, the anephric patients and the patients with a nonfunctioning renal allograft did not seem to be different from the uremic patients with native kidneys in situ. Cotes et al. (16) also reported t<sub>1/2</sub>...
values in four anephric patients that were at the same level as values found in the same study in uremic patients with preserved kidneys.

The basal urinary excretion of EPO is low in healthy persons, 0.9 to 2.8 U/day (8), and 4 U/day has been reported (7). After an iv injection of EPO in doses between 10 and 1,000 U/kg, less than 5% was excreted in the urine (26). In renal insufficiency, less than 3% of an iv dose of EPO was excreted in the urine (9). In nephrotic syndrome, a urinary excretion of EPO of 1.3 U/g of urinary creatinine has been reported (35). Although only a minor part of the EPO is excreted in the urine, the kidney may still contribute to the elimination of EPO by filtration and tubular degradation, as is the case with many other protein substances (36).

The observed abnormal metabolism of EPO was probably not due to toxins cleared at different rates in CAPD and HD because the pharmacokinetic parameters were similar. This is in accordance with an earlier observation (19). The peritoneal membrane seems to be poorly permeable to EPO, and our data confirm that the loss of EPO in the peritoneal fluid is insignificant during EPO treatment (20). The poor penetration of EPO has also been shown in the reverse direction by a low bioavailability of EPO administered in the dialysis fluid (20, 22).

The volume of distribution was comparable to the plasma volume in both the healthy individuals and the uremia patients. This is consistent with the other reports and indicates that most of the EPO is remaining free in the circulation (16, 27).

The estimated endogenous EPO production rate was based on the assumption that the C0 is representative for the steady-state native s-EPO and that the calculated clearance is valid for the native EPO in steady state. Our estimate in uremic patients of 146 U/day per 1.73 m² = 2.4 U/kg per day is comparable to 7 and 3 U/kg per day obtained in earlier reports of uremic patients (15, 16). In healthy volunteers, the endogenous basal production has been estimated to 3 U/kg per day (29) versus our value of 290 U/day per 1.73 m² = 4.6 U/kg per day. The lower estimated production rate of EPO in our patients was mainly due to the lower clearance because the level of s-EPO was close to the level in the healthy volunteers.

The endogenous EPO production rate has been measured by a constant infusion of [125I]EPO. In uremic patients, it was 255 pg/kg per hour, versus 190 pg/kg per hour in healthy control subjects (not significant). The uremic patients had a mean s-EPO level three times higher than the healthy controls (30.9 versus 11.1 pg/mL) (10). By use of the mean values given by the authors, the clearance in uremia = EPO production at steady state/EPO concentration at steady state = 8.25 mL/kg per hour versus 17.1 mL/kg per hour in the healthy individuals. It appears that the [125I]EPO tracer infusion study showed a reduced clearance of EPO in uremic patients, as we did by using therapeutic EPO doses.

We found a significantly lower absorption fraction or bioavailability in the uremic patients than in the healthy individuals. The site of injection may be of importance. The absorption of sc-injected EPO labeled with 125I may be better from the thigh compared.
with the arm and the abdominal skin (37). In uremic patients, the bioavailability of sc-administered EPO has been reported to be 24 to 49% after injection in the abdominal skin (18,19,22,24), 18 to 32% in the arm (20,21), and 22 to 44% at unspecified locations (23,25). In healthy persons, the figures are 32 to 36% after injection in the arm (27,29) and 15% at unspecified locations (28). Our findings were within the range of these reported values, but these studies did not directly compare uremic patients with healthy subjects, they used different injection sites, and in some of them, the monitoring period was too short. The number of healthy subjects completing the sc part of this study was reduced. In spite of this, the difference was still evident when subgroups were compared according to sex and age. The reason for this difference in absorption fraction is unknown. EPO has a molecular weight of about 30,400 (38), and a large part of the sc depot may be absorbed through the lymphatics (39). EPO may to some extent be trapped and metabolized in the regional lymph nodes. The absorption of EPO may accordingly depend on differences in lymph flow and physical activity (40). Another possibility is local enzymatic degradation, which has been proposed in some cases of insulin resistance (41). Uremia is characterized by metabolic acidosis. It may be speculated that this may change the charge properties and render it more prone to degradation. The perfusion of the skin and the nutritional status may also be important. The skinfold thickness has been shown to correlate with sc blood flow and to correlate with insulin absorption rate from the abdominal wall (42). However, no correlation between the thickness of the sc tissue assessed by ultrasound and the absorption of $^{[125]}$EPO has been found in healthy individuals (37). The patients were anemic. Whereas reports on elimination kinetics are contradictory, the absorption seems to be unchanged after correction of the anemia by regular EPO treatment (16,17,25). In Figure 2, the initial slope was steepest in the group of healthy subjects. This is apparently conflicting with the estimated rate of sc absorption, which was not different in the two groups. However, the initial slope in the sc curves is determined not only by the rate of absorption but also by absorption fraction, dose, and volume of distribution (Slope = $a$ x f x Dose/V). In this case, the difference can be explained by the higher absorption fraction (f) in the group of healthy subjects. The absorption was much slower than the elimination in both groups. This means that the terminal part of the sc curves is determined mainly by the rate of absorption because this is the rate-limiting step. Some of the sc curves had two peaks. This indicates an inconstant absorption. The result of the nonlinear regression analysis can therefore only be an approximation. The MIT calculated independently on the model may be preferable. It may be speculated that the absorption rate slows down at rest during the night. In the morning, muscular activity may accelerate the absorption, again resulting in a second peak. Hypothetically, there may also be a release of EPO accumulated as a depot in the regional lymph nodes.

In nonrenal anemia, the s-EPO is negatively correlated with hematocrit (43). We found a modest positive correlation between hematocrit and s-EPO in the uremic group. The significance of this is not clear. Our calculations are dependent on the assumption of a constant endogenous s-EPO level and production during the investigation period. A circadian variation has been observed in hospitalized patients with various diseases, with the lowest level at 8:00 a.m. and the highest level at 8:00 p.m., but the absolute difference was small compared with the levels recorded during this investigation (44). In normal individuals, no diurnal variation of s-EPO was found (45). A diurnal variation has not been described in uremic patients. It was concluded that uremia was associated with a reduced production rate of native EPO, a reduced bioavailability of sc-administered EPO, and a reduced clearance of exogenous EPO compared with healthy volunteers. The low clearance of EPO in uremia may help to preserve the basal levels of s-EPO, even in the presence of a reduced production of EPO.

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"Study with me then, a few things in the spirit of truth alone, so that we may establish the manner of Nature's operations . . . For this essay which I plan will shed light upon the structure of the kidney. Do not stop to question whether these ideas are new or old, but ask more properly, whether they harmonize with Nature. And be assured of this one thing, that I never reached my idea of the structure of the kidney by the aid of books, but by the long, patient and varied use of the microscope. I have gotten the rest by the deductions of reason, slowly, and with an open mind as is my custom."